Minireview

Decoding the Cardiac Actions of Protein Kinase D Isoforms

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ABSTRACT

Protein kinase D (PKD) consists of a family of three structurally related enzymes that play key roles in a wide range of biological functions that contribute to the evolution of cardiac hypertrophy and heart failure. PKD1 (the founding member of this enzyme family) has been implicated in the phosphorylation of substrates that regulate cardiac hypertrophy, contraction, and susceptibility to ischemia/reperfusion injury, and de novo PRKD1 (protein kinase D1 gene) mutations have been identified in patients with syndromic congenital heart disease. However, cardiomyocytes coexpress all three PKDs. Although stimulus-specific activation patterns for PKD1, PKD2, and PKD3 have been identified in cardiomyocytes, progress toward identifying PKD isoform-specific functions in the heart have been hampered by significant gaps in our understanding of the molecular mechanisms that regulate PKD activity. This review incorporates recent conceptual breakthroughs in our understanding of various alternative mechanisms for PKD activation, with an emphasis on recent evidence that PKDs activate certain effector responses as dimers, to consider the role of PKD isoforms in signaling pathways that drive cardiac hypertrophy and ischemia/reperfusion injury. The focus is on whether the recently identified activation mechanisms that enhance the signaling repertoire of PKD family enzymes provide novel therapeutic strategies to target PKD enzymes and prevent or slow the evolution of cardiac injury and pathological cardiac remodeling.

SIGNIFICANCE STATEMENT

PKD isoforms regulate a large number of fundamental biological processes, but the understanding of the biological actions of individual PKDs (based upon studies using adenoviral overexpression or gene-silencing methods) remains incomplete. This review focuses on dimerization, a recently identified mechanism for PKD activation, and the notion that this mechanism provides a strategy to develop novel PKD-targeted pharmaceuticals that restrict proliferation, invasion, or angiogenesis in cancer and prevent or slow the evolution of cardiac injury and pathological cardiac remodeling.

Introduction

Protein kinase D (PKD) consists of a family of three structurally related stress-activated enzymes (PKD1 or PKD1\(\mu\), PKD2, and PKD3 or PKD(C)) that regulate a large number of fundamental biological processes involved in cell proliferation, differentiation, apoptosis, immune regulation, cardiac contraction, cardiac hypertrophy, angiogenesis, and cancer (Harrison et al., 2006; Rozengurt, 2011; Ren, 2016; Roy et al., 2017; Simsek Papur et al., 2018; Youssef and Ricort, 2019; Renton et al., 2021; Zhang et al., 2021). The traditional model for PKD activation based upon a series of early studies from the Rozengurt laboratory holds that PKD isoforms are activated by receptor-driven pathways that promote diacylglycerol (DAG) accumulation and colocalize PKD with its upstream activator protein kinase C (PKC) at the plasma membrane (Rozengurt et al., 2005). This facilitates PKC-dependent transphosphorylation of PKD at conserved serine residues in the activation loop (AL). However, it is now evident that PKDs can also be activated through a PKC-independent AL autophosphorylation mechanism (Jacamo et al., 2008; Rybin et al., 2009; Steinberg, 2012), PKDs are activated via a distinct tyrosine phosphorylation-dependent mechanism during oxidative stress (Storz et al., 2003; Storz et al., 2008; Youssef and Ricort, 2019; Renton et al., 2021; Zhang et al., 2021). The traditional model for PKD activation based upon a series of early studies from the Rozengurt laboratory holds that PKD isoforms are activated by receptor-driven pathways that promote diacylglycerol (DAG) accumulation and colocalize PKD with its upstream activator protein kinase C (PKC) at the plasma membrane (Rozengurt et al., 2005). This facilitates PKC-dependent transphosphorylation of PKD at conserved serine residues in the activation loop (AL). However, it is now evident that PKDs can also be activated through a PKC-independent AL autophosphorylation mechanism (Jacamo et al., 2008; Rybin et al., 2009; Steinberg, 2012), PKDs are activated via a distinct tyrosine phosphorylation-dependent mechanism during oxidative stress (Storz et al., 2003; Storz et al., 2008; Youssef and Ricort, 2019; Renton et al., 2021; Zhang et al., 2021). This work was supported by National Institutes of Health National Heart, Lung, and Blood Institute [Grant RO1-HL112338].

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ABBREVIATIONS: \(\alpha_{1}-AR\), \(\alpha_{1}\)-adrenergic receptor; AKAP, A-kinase anchoring protein; AL, activation loop; Bcl-2, B-cell lymphoma-2; CHD, congenital heart disease; CREB, cyclic-nucleotide regulatory element binding protein; cTnI, cardiac troponin I; DAG, diacylglycerol; DLP1, dynamic-like/related protein 1; HDAC, histone deacetylase; LIMK, LIM kinase; LPL, lipoprotein lipase; mAKAP, muscle AKAP; MEF2, myocyte enhancer factor 2; MnSOD, manganese superoxide dismutase; NF-xB, nuclear factor \(\alphaB\); nPKC, novel PKC isoform; NTDD, N-terminal dimerization domain; PDZ, PSD-95/disks large/zona occludens; PH, pleckstrin homology; PKA, protein kinase A; PKC, protein kinase C; PKD, protein kinase D; Rho, rat sarcoma virus homolog; RhoA, Rho family member A; ROS, reactive oxygen species; S1P, sphingosine 1-phosphate; SSH1L, slingshot 1L.
PKD Activation Mechanisms and Actions in Cardiomyocytes

PKD Structure; the Canonical Mechanism for PKD Activation. PKD isoforms share a common modular domain structure consisting of a C-terminal kinase domain and an N-terminal regulatory domain (Fig. 1). Studies to date have focused on the tandem C1A/C1B motifs in the regulatory domain that anchor full-length PKD to lipid membranes and the pleckstrin homology (PH) motif that participates in an intramolecular autoinhibitory interaction that regulates enzyme activity (Rozengurt et al., 2005; Steinberg, 2012). The classic model for PKD activation involves receptor-dependent pathways that promote DAG accumulation and colocalize PKD with allosterically activated novel protein kinase C isoforms (nPKCs) at the plasma membrane, leading to nPKC-dependent trans-phosphorylation of PKD at conserved serine residues in the AL. Activated forms of PKD1, PKD2, and PKD3 then phosphate target substrates. PKD1 and PKD2 also autophosphorylate at a PKD consensus phosphorylation motif [LxxRxS/T, in a PSD-95/disk/zoa occludins (PDZ) domain-binding motif] at the extreme C terminus—this modification regulates PKD1/2 interactions with PDZ domain-containing scaffolding proteins, PKD1/2 trafficking, and the amplitude/tempo of PKD1/2 signaling responses (Sanchez-Ruiloba et al., 2006; Kunkel et al., 2009). PKD3 lacks a C-tail autophosphorylation site and is not regulated in this manner.

PKC-Independent AL Autophosphorylation. PKD activation is typically associated with an increase in AL phosphorylation, a post-translational modification originally ascribed to an nPKC-dependent mechanism based upon a large number of studies showing that AL phosphorylation is effectively blocked by PKC inhibitors that do not directly inhibit PKD activity (Fig. 2). However, it is worth noting that there is only very limited direct in vitro experimental evidence that nPKC isoforms actually phosphorylate PKD1 at its AL sites (at Ser744 and Ser748) and Ser749 nomenclature based upon rodent sequence corresponding to Ser744 and Ser748 in human PKD1. Our current understanding of the mechanisms that regulate AL phosphorylation is also confounded by the fact that most studies have relied on a phosphorylation state–specific antibody labeled anti–PKD1-pS744/pS748 that actually tracks phosphorylation primarily at Ser744 (not Ser748). With the development of a different phosphorylation state–specific antibody that specifically recognizes Ser748 phosphorylation, it became apparent that the mechanisms and consequences of PKD1 phosphorylation at Ser744 versus Ser748 differ. Specifically, cell-based studies show that the catalytically inactive PKD1 mutant enzyme is phosphorylated at Ser744 but not at Ser748 in cells treated with Phorbol 12-myristate 13-acetate; this result argues that kinase-dead PKD1 is phosphorylated at Ser744 in trans by an endogenous cellular Ser/Thr kinase (namely PKC) and that PKD1-Ser748 phosphorylation in cells requires an autophosphorylation reaction that is defective in the catalytically inactive enzyme (Rybin et al., 2009). Conversely, the constitutively active PKD1-ΔPH deletion mutant (lacking the PH domain) is recovered from COS-7 cells with a high level of AL phosphorylation at Ser748 (but not at Ser744) even when cells are treated with a pan-PKC inhibitor (Rybin et al., 2009). These results indicate
that PKD1-ΔPH-Ser^748 phosphorylation is attributable to a PKC-independent autocatalytic reaction. It is worth noting that this particular feature of PKD regulation is evolutionarily conserved; the AL sequence of DFK-1 (the Caenorhabditis elegans PKD homolog) contains a single phospho-acceptor site at QFRKT^588 (corresponding to Ser^748 in PKD1) that is a target for a PKC-independent autocatalytic reaction (Feng et al., 2006).

There has been some progress in defining the molecular controls for AL autophosphorylation. We showed that a Ser-Ala substitution at the C-tail autophosphorylation site (at Ser^916) prevents PKD1 autophosphorylation at Ser^748 (i.e., the major AL autotyrosine kinase

...an activation loop phosphorylation. It is worth noting that this particular feature of PKD regulation is evolutionarily conserved; the AL sequence of DFK-1 (the Caenorhabditis elegans PKD homolog) contains a single phospho-acceptor site at QFRKT^588 (corresponding to Ser^748 in PKD1) that is a target for a PKC-independent autocatalytic reaction (Feng et al., 2006). The redox-dependent mechanism for PKD1 activation (the isoform most intensively studied in this context) involves a hierarchical series of phosphorylations involving c-Abl and Src (two nonreceptor tyrosine kinases that are activated by oxidative stress): c-Abl-dependent phosphorylation at Y^463 in the PH domain leads to a conformational change that is permissive for Src-mediated phosphorylation at Y^66 in the N terminus. pY^95 serves as a docking site for the PKCδ-C2 domain, which then phosphorylates PKD1's AL at Ser^738/Ser^742 (Storz et al., 2003; Storz and Toker, 2003b; Doppler and Storz, 2007). Although reactive oxygen species (ROS)–activated PKD1 has been implicated in the activation of the apoptosis signal-regulating kinase 1–c-Jun N-terminal kinase pathway (a cell death pathway (Zhang et al., 2005a)), and a previous study from our laboratory implicated PKD1 in a H_2O_2-activated pathway that decreases the abundance of the transcription factor cyclic-nucleotide regulatory element binding protein (CREB), thereby disrupting Cre-dependent gene expression in cardiomyocytes (Ozgen et al., 2009), most studies (performed in noncardiomyocytes) have focused on ROS-activated PKD1 localization to mitochondria where it triggers a mitochondrion-to-nucleus signaling pathway involving nuclear factor κB (NFκB). This PKD1-NFκB pathway induces expression of antioxidant/anti-apoptotic genes, such as manganese superoxide dismutase (MnSOD), that detoxify cellular ROS and promote cell survival (Storz and Toker, 2003a; Storz et al., 2005). Notably, the canonical growth factor–dependent pathway for PKD1 activation does not decrease CREB protein abundance, stimulate NFκB, or induce MnSOD. These results emphasized that 1) ROS-activated PKD1 can serve as a nodal point in signaling networks that trigger either prosurvival or proapoptotic responses and 2) the signaling repertoire and cellular actions of PKD1 are highly contextual.

**PKD2.** Tyrosine phosphorylation and PKCδ also are required for ROS-dependent activation of PKD2, but the mechanism differs. Here, an initial PKCδ-dependent phosphorylation of AL serine residues primes the enzyme for subsequent c-Abl–dependent phosphorylation at Y^717 in the activation segment P^+1 loop (a sequence that is highly conserved in all PKD isoforms but phosphorylated only in PKD2); the PKCδ-PKD2 interaction does not require phosphorylation of the conserved putative PKCδ-C2 domain-docking site (Y^67) in PKD2's N terminus (Cobbaut et al., 2017). Redox-activated PKD2 contributes to NFκB activation but via a mechanism that does not require PKD2 kinase activity (Mihailovic et al., 2004; Cobbaut and Van Lint, 2018). Rather, PKD2's kinase-independent role has been attributed to the formation of PKD1/PKD2 heterodimers, with PKD2 acting as a scaffold to facilitate PKD1 activation (either by trapping activators, such as PKCδ, Abl, and Src, or by protecting PKD1 from phosphatase inactivation). Although the Y^67 phosphorylation site in the PKD2 N terminus is not required for ROS-dependent PKD2 activation, Y^67 phosphorylation (via a rat sarcoma virus homolog (Rho) family member A (RhoA)–Src pathway) is required for PKD2 localization to focal adhesions, PKD2 interaction with focal adhesion kinase, and PKD2 regulation of cell adhesion/migration (Durand et al., 2017). Notably, PKD1 and PKD2 N-terminal tyrosine phosphorylation sites sit in close proximity to the recently described dimerization interface (vide infra); the notion that N-terminal tyrosine phosphorylation interferes with PKD dimerization has not been considered.

**PKD3.** There is a limited amount of evidence for redox-regulation of PKD3. Specifically, our studies using Mn^{2+}-Phos-tag sodium dodecyl sulfate polyacrylamide gel electrophoresis (a method that exaggerates phosphorylation-dependent mobility shifts) show that H_2O_2 activates all three PKD isoforms (PKD1, PKD2, and PKD3) in cardiomyocytes (Qiu and Steinberg, 2016). The structural basis for ROS-dependent activation of PKD3 remains uncertain since PKD3 contains a Phe in place of Tyr at the putative N-terminal PKCδ-C2 domain-docking site.
PKD1 Cleavage by Caspase-3 in the Setting of Apoptosis. Caspase-3 cleaves PKD1 at a site in the C1-PH inter-domain (that is conserved in PKD1 but not PKD2 or PKD3), generating a catalytic fragment that retains the autoinhibitory PH but not the C1 domain (Endo et al., 2000). The functional consequences of caspase-3–dependent PKD1 cleavage have been disputed. Although early studies concluded that PKD1 is a proteolytically activated enzyme, this was based on in vitro kinase assays showing that the PKD1 C-terminal cleavage product generated during apoptosis displays enhanced lipid-independent phosphorylation of a peptide substrate (Haußermann et al., 1999; Endo et al., 2000). Studies from our laboratory challenged this conclusion by showing that peptide kinase assays do not necessarily provide valid surrogate readouts of PKD1 activity toward physiologically relevant protein substrates (Rybin et al., 2009). We showed that the PKD1-Δ1–321 truncation mutant (which models the C-terminal fragment generated as a result of caspase-3 cleavage) displays constitutive lipid-independent S916, autocatalytic activity, and it phosphorylates small peptide substrates (such as CREB-tide), but it does not autophosphorylate at its AL or phosphorylate protein substrates, such as CREB or cardiac troponin I (Rybin et al., 2012). Cell-based studies provide further evidence that PKD1 cleavage provides a mechanism to limit its activity, showing that PKD1-Δ1–321 does not substitute for wild–type PKD1 as an in vivo activator of the CREB or extracellular signal-regulated–kinase phosphorylation pathways (Rybin et al., 2012). Other studies showing that PKD1′s actions to regulate lipoprotein lipase (LPL)–mediated triglyceride accumulation in cardiomyocytes are lost in the diabetic heart as a result of activation of caspase-3 and caspase-3–dependent cleavage/inactivation of PKD1 lend further support to the notion that caspase-3–dependent cleavage provides a mechanism to inhibit PKD1 (Kim et al., 2009).

What is the significance of the contextual differences in PKD isoform activation? The conventional allosteric model for PKD activation by lipid cofactors—which focuses on translocation events that deliver the enzyme in an active conformation to target substrates in membranes—assumes that PKD substrates are restricted to DAG-enriched membranes. This common stereotypical model for PKD activation does not explain observed differences in the subcellular compartmentalization patterns and spatiotemporal regulation of individual PKD isoforms that have been described in various cell types, and it does not adequately explain PKD phosphorylation of substrates in other cellular compartment, such as mitochondria, nucleus, or sarcomere (Rey et al., 2001, 2003; Bossuyt et al., 2011). The conventional allosteric model for PKD activation by lipid cofactors also assumes that PKD activity is an inherent/immutable property of the enzyme that is not altered by the activation process. However, there is growing evidence that stimulus-induced changes in phosphorylation can alter the enzymology (activity, cofactor requirements, or evidence substrate specificity) of certain protein kinases. We previously described such a mechanism for a different lipid-regulated protein kinase, namely PKCδ. We identified differences in the enzymology of the phorbol 12-myristate 13-acetate versus the redox-activated PKCδ enzyme; PKCδ becomes a lipid-independent kinase with altered substrate specificity when tyrosine is phosphorylated by Src (Steinberg, 2015). In this context, it is interesting to note that there is only very limited information on the enzymology of redox-activated (Tyr-phosphorylated) PKDs. Available studies have not considered whether the redox mode for PKD activation (which is accompanied by a distinct set of phosphorylations on the enzyme) “fine-tunes” PKD′s activity. Context-dependent changes in PKD′s enzymology could underlie observed differences in the signaling properties of the redox-activated PKD1 versus PKD2 enzymes and the observation that PKD2 phosphorylates the interferon–α/β receptor chain 1 at QTSQDps^656—a site that does not conform to the PKD LxRxRxxpS/T consensus phosphorylation motif and is not phosphorylated by PKD1 or PKD3 (Zheng et al., 2011). Finally, it is worth noting that the Newton laboratory has taken advantage of genetically encoded fluorescence resonance energy transfer–based kinase activity reporters for PKD to identify differences in the spatiotemporal control of PKD activation at Na+/H+ exchanger regulatory factor 1 (the PDZ domain-containing scaffolding protein that interacts with PKD1 and PKD2) when compared with PKD activity in the bulk cytosol or at the plasma membrane; the phorbol ester (Phorbol 12,13-dibutyrate) or receptor (histamine) that evoked increases in PKD activity at Na+/H+ exchanger regulatory factor 1 are more rapid and sustained (although decrease in overall amplitude) when compared with the PKD activation profiles in the other cellular compartments (Kunkel et al., 2009). These studies raise the intriguing notion that other mechanisms for PKD activation (during oxidative stress or as a result of proteolytic cleavage) might also lead to the accumulation of pools of enzymes with distinct catalytic properties and that PKD activation signatures are stimulus-specific. Given that signaling output reflects the ensemble effects of both the individual PKD isoforms coexpressed by a particular cell type and their distinct activation profiles in different subcellular compartments, such a mechanism would vastly increase the signaling repertoire of PKD family enzymes.

PKD Dimerization. There is evidence that PKDs (in most cases, PKD2 and PKD3) activate effector responses, such as protein transport, from the trans-Golgi network to the cell surface and F-actin–directed cell motility as dimers (Bosnard et al., 2007; Doppler et al., 2014); PKD2 is also reported to play a kinase-independent role (in heterodimeric complexes with PKD1) in ROS-dependent activation of NFκB (Mihailovic et al., 2004; Cobbett and Van Lint, 2018). However, the notion that dimerization plays a more general role to regulate PKD activity (and the identification of the structural basis for PKD dimerization) is quite recent and is summarized in this section.

The Rubin laboratory identified a highly conserved 92-amino-acid–sequence N-terminal to the C1α DAG-binding site of mammalian PKD1-2 and Dkk-2A (a C. elegans PKD homolog) that functions independently of other regulatory or catalytic domain regions to direct PKD monomer incorporation into homodimeric or heterodimeric complexes (Fig. 1) (Aicart-Ramos et al., 2016). Importantly, this study also demonstrated that overexpression of the isolated N-terminal dimerization domain (NTDD) is sufficient to disrupt PKD dimerization, PKD phosphorylation of a cellular substrate...
PKD Isoform-Specific Functions in Cardiomyocytes

PKD Isoforms Are Activated in an Isoform-Specific Manner in Cardiomyocytes. Cardiomyocytes coexpress PKD1, PKD2, and PKD3. Although there is evidence that some cellular responses are activated in a functionally redundant fashion by multiple PKDs, studies from our laboratory expose a mechanism for isoform specificity showing that PKD isoforms are activated in an agonist- and isoform-specific manner in neonatal cardiomyocyte cultures. Specifically, we showed that β₂-adrenergic receptor (β₂-AR) agonists specifically activate PKD1 (not PKD2 or PKD3), whereas the protease-activated receptor agonist thrombin or the endothelial differentiation gene–receptor agonist sphingosine 1-phosphate (S1P) preferentially activate PKD2 and PKD3 (Fig. 4) (Guo et al., 2011; Qiu and Steinberg, 2016). Inhibitor studies link the β₂-AR–PKD1 pathway to increased phosphorylation of CREB, an increase in CRE-dependent gene expression, and induction of B-cell lymphoma-2 (Bel-2) [a CREB target gene; i.e., PKD1 links β₂-AR activation to CREB phosphorylation in the S1⁵³³ PKD consensus phosphorylation motif (Guo et al., 2011)]. In contrast, thrombin and S1P activate a different Rho-dependent pathway that preferentially activates PKD2/PKD3 and promotes CREB-S¹⁵³³ phosphorylation; Rho is not required for β₂-AR–dependent PKD1-CREB-S¹⁵³³ phosphorylation (Qiu and Steinberg, 2016).

Fig. 3. Schematic of the oxidative stress pathways that activate PKD isoforms and the PKD-dependent mechanisms that regulate apoptosis. Oxidative stress activates PKD through a mechanism involving coordinate phosphorylations by the redox-activated nonreceptor tyrosine kinases c-Abl, Src, and PKC. Although ROS-activated PKD1 has been implicated in a pathway that induces expression of antioxidant/antiapoptotic genes, such as MnSOD, which detoxify cellular ROS and promote cell survival, PKD also has been implicated in a mechanism that regulates cofilin phosphorylation by the protein phosphatase SSH1L; this mechanism prevents cofilin interaction with the proapoptotic molecule Bax, and confers cardioprotection. PKD also has been implicated in an β₂-AR–dependent pathway that leads to phosphorylation of the mitochondrial fission protein DLPL that localizes to the mitochondrial membrane, triggers ROS generation, and induces cellular apoptosis. PKD and proteins known to be direct substrates of PRD are shaded pink. IKK, IκB kinase.
The observation that Rho recruits a signaling pathway that is specific for PKD2/PKD3 resonates with a previous study from the Storz laboratory implicating PKD2/PKD3 complexes in the Rho-dependent pathway that regulates cardiac remodeling and cell migration (Doppler et al., 2014).

**Hypertrophy/Heart Failure.** Early studies focused on PKD1’s role in pathological cardiac remodeling, which showed that PKD1 knockdown attenuates agonist-induced hypertrophy of cultured cardiomyocytes, that cardiac-specific PKD1-S744E overexpression induces hypertrophy that transitions to cardiac fibrosis (Harrison et al., 2006; Fieltz et al., 2008). These cardiac actions of PKD have been attributed to phosphorylation of histone deacetylase (HDAC) 5, a class IIa histone deacetylase that acts as an inhibitory regulator of myocyte enhancer factor 2 (MEF2). PKD-dependent HDAC5 phosphorylation at S239/S498 creates docking sites for 14-3-3 proteins that escort HDAC5 from the nucleus, freeing nuclear MEF2 to activate pathological gene programs (Fig. 5). Although all three PKD isoforms possess in vitro HDAC5 kinase activity, studies to date have focused on the cellular actions of PKD1, showing that small interfering RNA knockdown of PKD1 blunts (but does not fully abrogate) receptor-dependent HDAC5 phosphorylation (Vega et al., 2004; Huynh and McKinsey, 2006; Matthews et al., 2006). These results could suggest that PKD isoforms regulate HDAC5 in a functionally redundant manner, although the alternative interpretation—that this is regulated by PKD dimers—is also possible and has never been considered.

There is evidence that the PKD1/HDAC5/MEF2 pathway is amplified through interactions with scaffolding proteins, such as Δ-kinase anchoring protein (AKAP)-Lbc or the muscle Δ-kinase anchoring protein β (mAKAP/β), two scaffolds that serve as platforms to coordinate PKD activation by PKC (Diviani et al., 2001; Carnegie et al., 2004, 2008; Kritzer et al., 2014; Dodge-Kafka et al., 2018). AKAP-Lbc is expressed at high levels in cardiomyocytes, is upregulated in response to hypertrophic stimuli, and is unique among AKAPs in that it has Rho–guanine nucleotide exchange factor activity; it enhances signaling efficiency through the PKD/HDAC5/MEF2 pathway that activates the fetal gene program (Diviani et al., 2001; Carnegie et al., 2004, 2008). Similarly, mAKAP/β is a muscle-specific scaffolding protein that serves as a platform to organize a signaling module involving PKD1, its upstream activator phospholipase Cε [which generates a local pool of DAG from phosphatidylinositol 4-phosphate in the perinuclear Golgi apparatus (Zhang et al., 2013)], and HDAC4/5; targeted deletion of mAKAP/β disrupts in vivo pressure overload–induced PKD activation and PKD-dependent HDAC4/5 phosphorylation (Kritzer et al., 2014; Dodge-Kafka et al., 2018). Studies to date have focused on AKAP-Lbc or mAKAP/β as scaffolds for PKD1. The notion that these proteins also bind/scaffold PKD2/3 has never been considered.

Finally, PKD also phosphorylates CREB, a basic leucine zipper transcription factor that binds cyclic-nucleotide regulatory elements in the regulatory region of CREB target genes and contributes to the maintenance of normal ventricular structure and function (Fentzke et al., 1998; Ozgen et al., 2008). PKD-dependent CREB-S133 phosphorylation leads to the recruitment of coactivators (such as CREB-binding protein/p300, which is also a PKD substrate) and increased expression of CREB target genes, including Bcl-2, atrial natriuretic factor, and brain natriuretic peptide (Zhang et al., 2005b). We previously showed that z1-ARs activate a PKCα-PKD pathway leading to CREB-S133 phosphorylation, increased Cre-dependent gene expression, and induction of Bcl-2 in cardiomyocytes (Ozgen et al., 2008). However, the relative importance of PKD-dependent pathways involving CREB versus HDAC5 in adverse cardiac remodeling has never been directly examined and remains uncertain.

**Phosphorylation of Sarcomeric Proteins.** PKD regulates cardiac myofilament function (and thereby contraction)
through the coordinated phosphorylation of multiple sarcomeric proteins at sites that are also targets for phosphorylation by protein kinase A (PKA). PKD decreases myocardial Ca\(^{2+}\) sensitivity by phosphorylating cardiac troponin I (cTnI) at Ser\(^{22/23}\) (or Ser\(^{23/24}\), if the initiating methionine is counted), and it accelerates crossbridge cycle kinetics at least in part by phosphorylating the thick filament protein cardiac myosin binding protein-C at Ser\(^{302}\) (Bardswell et al., 2010; Martin-Garrido et al., 2018). Notably, these PKD phosphorylation sites also are targets for phosphorylation by PKA, fueling speculation that PKD-dependent regulation of cardiac contractility may assume greater functional significance in settings, such as heart failure, wherein the PKA pathway is downregulated. Moreover, although PKA phosphorylates cTnI at both Ser\(^{22}\) and Ser\(^{23}\), the prevailing notion that phosphorylation at both sites is required to decrease myofibrillar Ca\(^{2+}\) sensitivity was challenged by studies from the Avkiran and Kentish laboratories showing that PKD phosphorylates cTnI primarily at Ser\(^{23}\) and this PKD-dependent monophosphorylation of cTnI is sufficient to decrease myofibrillar Ca\(^{2+}\) sensitivity (Martin-Garrido et al., 2018). Again, all three PKD isoforms share this in vitro kinase activity, but their individual roles in the regulation of contraction and the notion that a dimerization mechanism may influence sarcomeric protein phosphorylation have never been considered.

**Cardiac Adaptation to Metabolic Derangements in the Diabetic Heart.** PKDs regulate vesicles fission from Golgi membranes and thereby control trafficking of LPL to surface membranes (Kim et al., 2008). In the setting of moderate hypoinsulinemia (with impaired glucose uptake and glycolysis), a PKD-dependent pathway that requires the formation of a functional complex between PKD, LPL, and glycolysis), a PKD-dependent pathway that requires the for-

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erate hypoinsulinemia (with impaired glucose uptake and surface membranes (Kim et al., 2008). In the setting of mod-

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eukaryotic protein kinases and plays a critical role to bind and structural data suggest that other residues at this position

S189

S592

S637

which involves caspase-3–dependent cleavage and inactivation of PKD1 (the only isoform that contains a caspase-3 cleavage site)—functions to limit PKD-mediated delivery of LPL to the surface membrane and prevent cardiac triglyceride overload (Kim et al., 2009).

**PKD Isoforms and Cardiac Injury Versus Cardioprotection.** PKDs have been implicated in both ischemia/reperfusion injury and cardioprotection. Studies from our laboratory show that oxidative stress (a stimulus that plays an important role in the evolution of heart failure syndromes) leads to the activation of PKD1, PKD2, and PKD3 in cardiomyocytes and cardiac fibroblasts (Qiu and Steinberg, 2016) and that H\(_2\)O\(_2\)–dependent activation of PKD1 leads to a decrease in the abundance of CREB (a transcription factor that is important for the maintenance of normal physiologic function) in cardiomyocytes (Ozgen et al., 2009). However, other studies implicate PKD in an S1P receptor pathway that regulates coflin phosphorylation and confers cardioprotection. The PKD-dependent pathways that regulate coflin (a mechanism investigated most comprehensively in studies that interrogate coflin’s role as an actin-binding protein that regulates of cell motility and migration) are described below.

Coflin’s role in the regulation of cell migration is controlled through a complex/elaborate set of phosphorylations. Coflin phosphorylation by LIM kinase (LIMK) leads to a decrease in cell migration, whereas cell migration is restored when coflin is dephosphorylated by the protein phosphatase slingshot 1L (SSH1L). Importantly, SSH1L is a substrate for (and is negatively regulated by) PKD. PKD-phosphorylated SSH1L interacts with 14-3-3 adaptor proteins that sequester SSH1L in the cytosol away from coflin and F-actin (Niwa et al., 2002; Eiseler et al., 2009; Peterburs et al., 2009) (see Figs. 2 and 4). However, PKDs can also increase coflin phosphorylation by phosphorylating and activating p21-activated kinase, an upstream activator of LIMK (Spratley et al., 2011). Studies in cardiomyocytes have focused on the S1P-RhoA

PKD signaling pathway; this pathway inactivates SSH1L, increases coflin phosphorylation and thereby prevents coflin translocation to mitochondria, prevents the coflin interaction with the proapoptotic molecule Bax, and blocks redox-induced cell death (see Fig. 2) (Chua et al., 2003; Xiang et al., 2013). Although this pathway would confer cardioprotection, there is also evidence that this mechanism might be counter-balanced by an \(z_1\)-AR–PKD pathway that leads to phosphory-

lation of the mitochondrial fission protein dynamic-like/related protein 1 (DLPL, also known as DRP-1) at its PKD consensus site (S\(^{537}\); PKD-phosphorylated DLPL is localized to the outer mitochondrial membrane where it triggers ROS generation, activates the mitochondrial permeability transition pore, promotes mitochondrial fragmentation, and activates apoptotic signaling (cytochrome c release and caspase cleavage) (Jhun et al., 2018). In theory, these results could suggest that PKD might play opposing roles to fine-tune the cardiomyocyte apoptotic response through the S1P receptor pathway that confers cardioprotection and the \(z_1\)-AR pathway that promotes apoptosis.

**PRKD1 Mutations and Syndromic CHD.** Genome-wide association studies link the PRKD1 gene to elevated body mass index (a cardiovascular risk), and PRKD1 has also been implicated in certain forms of syndromic CHD (Speliotes et al., 2010; Graff et al., 2013; Shaheen et al., 2015; Sifrim et al., 2016; Alter et al., 2021). Shaheen et al. (2015) implicated a homozygous truncation mutation in PRKD1 that results in the generation of a catalytically inactive protein (containing the entire N-terminal regulatory domain but only the first 35 residues of the kinase-domain N terminus) in truncus arteriosus. Sifrim et al. (2016) and our group identified heterozygous de novo missense mutations in PRKD1 in five patients with syndromic CHD associated with skin and limb abnormalities and developmental delay (Alter et al., 2021). Four of the five missense mutations map to the kinase domain. The implicit assumption of previous studies (which did not perform biochemical assays) was that disease-causing de novo PRKD1 mutations would be inactivating; this assumption was based largely on literature showing that homozygous PRKD1 knockout in mice is embryonic lethal (Fielitz et al., 2008). However, we showed that the kinase-domain R603H substitution identified in one patient is a gain-of-function mutation; PKD1-R603H displays high levels of constitutive/lipid-independent autocatalytic and CREB kinase activity (Alter et al., 2021). The G592R mutation identified in the three other patients is at the second glynine in the highly conserved Gly-rich loop (or G-loop), a residue conserved in more than 99% of eukaryotic protein kinases and plays a critical role to bind and orient ATP in an optimal position for catalysis (Hemmer et al., 1997; Steinberg, 2018). Although modeling studies based upon structural data suggest that other residues at this position
would result in steric clash and disrupt catalytic activity (Hemmer et al., 1997), we showed that PKD1-G592R is recovered from human embryonic kidney 293 cells with a considerable amount of lipid-dependent autocatalytic activity and reduced (but not absent) CREB-Ser133 kinase activity (Alter et al., 2021). These results emphasize that phenotypes described in gene knockout models (in which there is a loss of protein and activity) may not be relevant to heterozygous CHD-causing PKD1 truncation or missense mutations wherein there is a change in enzyme activity but preserved full-length or truncated protein (protein that can still exert a kinase-independent function in PKD homodimers or heterodimers or other multi-protein complexes involving PKD).

Conclusions and Clinical Implications

In the years since PKDs first emerged as signaling enzymes that play pivotal roles in biological mechanisms that influence cell growth, cell differentiation, proapoptotic and survival pathways, and angiogenesis, PKDs have been implicated in the pathogenesis of many inflammatory and metabolic disorders, the evolution of cardiac hypertrophy, ischemia/reperfusion injury and heart failure syndromes, and the pathogenesis and progression of many cancers. The evidence that PKD isoform expression is disregulated in many cancers with PRKD1 gene mutation identified in over 2.2% of cancer samples in The Cancer Genome Atlas and high frequency somatic mutations in PRKD genes identified in polymorphous low-grade adenocarcinoma and in angiolipomas (Weinreb et al., 2014) has provided a rationale to use high-throughput screens to develop compounds that interdict signaling by PKDs. Several laboratories have identified potent, selective, small-molecule pan-PKD inhibitors that show considerable promise in preclinical cancer models (Sharlow et al., 2008; Guha et al., 2010; Lavalle et al., 2016; Meredith et al., 2010; Monovich et al., 2010; Gamber et al., 2011; Roy et al., 2017). However, newer insights into the mechanisms and actions of PKD family enzyme that are summarized in this review raise certain caveats that must be considered in assessing the efficacy and safety associated with the use of pan-PKD inhibitors. First, cells typically express multiple PKD family members. Although PKD isoforms exhibit some functionally redundant actions, this review describes a number of functionally important differences in the activation mechanisms and signaling repertoires for individual PKD isoforms. A pan-PKD inhibitor may be of limited clinical utility in conditions in which PKD1 and PKD2/3 exert antithetical actions, such as in certain cancers (including breast, prostate, and gastric cancer) in which PKD1 acts as a tumor suppressor and PKD2/PKD3 increase proliferation, invasion, and chemoresistance. A similar limitation may apply to the use of inhibitors that lack isoform specificity to prevent ischemia/reperfusion injury or adverse cardiac remodeling, in which differences in the activation patterns and cellular actions of PKD1 versus PKD2/3 in cardiomyocytes have been identified. These concerns provide a strong rationale for the development of isoform-selective PKD inhibitors.

It is also worth emphasizing that PKD-targeted pharmacologicals that restrict proliferation, invasion, or angiogenesis in cancer might be predicted to have clinically important “off-target” effects by inhibiting PKD-dependent prosurvival pathways in other tissues. The field of cardio-oncology has emerged in recent years in response to growing concerns that cancer therapies that promote tumor cell apoptosis also limit prosurvival pathways in cardiomyocytes and lead to a cardio-toxicity that limits their use in a clinical setting (Sheng et al., 2016). This issue also much be considered.

Finally, this review focuses on the newer concept that a dimerization mechanism is required for at least certain PKD-driven responses. The mapping of a functionally important PKD dimerization interface provides the basis for future studies that use high-throughput screening and structure-based drug design to develop peptide inhibitors of this protein-protein interaction, compounds that can be exploited as research tools and could serve as prototypes for future therapeutics.

Authorship Contributions

Participated in research design: Steinberg.

Performed data analysis: Steinberg.

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References


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